J Food Sci Technol (February 2018) 55(2):811–820
https://doi.org/10.1007/s13197-017-3006-9

ORIGINAL ARTICLE

# Optimization of lipid profile and hardness of low-fat mortadella following a sequential strategy of experimental design

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Revised: 5 September 2017 / Accepted: 18 December 2017 / Published online: 5 January 2018


**Abstract** This study aims to optimize simultaneously the lipid profile and instrumental hardness of low-fat mortadella. For lipid mixture optimization, the overlapping of surface boundaries was used to select the quantities of canola, olive, and fish oils, in order to maximize PUFAs, specifically the long-chain n-3 fatty acids (eicosapentaenoic—EPA, docosahexaenoic acids—DHA) using the minimum content of fish oil. Increased quantities of canola oil were associated with higher PUFA/SFA ratios. The presence of fish oil, even in small amounts, was effective in improving the nutritional quality of the mixture, showing lower n-6/n-3 ratios and significant levels of EPA and DHA. Thus, the optimal lipid mixture comprised of 20, 30 and 50% fish, olive and canola oils, respectively, which present PUFA/SFA (2.28) and n-6/n-3 (2.30) ratios within the recommendations of a healthy diet. Once the lipid mixture was optimized, components of the pre-emulsion used as fat replacer in the mortadella, such as lipid mixture (LM), sodium alginate (SA), and milk protein concentrate (PC), were studied to optimize hardness and springiness to target ranges of 13–16 N and 0.86–0.87, respectively. Results showed that springiness was not significantly affected by these variables. However, as the concentration of the three components increased, hardness decreased. Through the desirability function, the optimal proportions were 30% LM, 0.5% SA, and 0.5% PC. This study showed that the pre-emulsion decreases hardness of mortadella. In addition, response surface methodology was efficient to model lipid mixture and hardness, resulting in a product with improved texture and lipid quality.

**Keywords** Response surface methodology (RSM) · Mixture design · Fatty acid profile · Hardness · Springiness · Mortadella

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## Introduction

Meat and meat products are fundamental elements in the human diet, providing essential nutrients, such as protein, fat, vitamins, and minerals. However, from the health perspective, high intake of these products is not recommended, mainly due to their high content of saturated fat (Jiménez-Colmenero 2007).

Overconsumption of saturated fat is associated with the occurrence of some diseases, such as obesity, colorectal cancer, and coronary heart disease (Zyriax and Windler 2000; Reddy 2002). Therefore, continuous efforts have been made by the food industry and food practitioners to develop new meat products with reduced fat levels, in order to suit the recommendations of the World Health Organization (WHO). According to the WHO, the ideal fat intake should represent between 15 and 35% of the total diet energy, and no more than 10% of the calorie intake should come from saturated fatty acids (SFA). Polyunsaturated fatty acids (PUFA) should represent between 6 and 11% (n-6, 2.5–8%; n-3, 0.5–2%) of the total calorie intake,

while monounsaturated fatty acids (MUFA) should account for between 10 and 15%, and trans fatty acids should represent no more than 1% (WHO 2003; Jiménez-Colmenero 2007).

Mortadella is a highly consumed and enjoyed emulsified meat product containing up to 30% fat (Jiménez Colmenero 2000), with predominance of saturated (9.5%) and monounsaturated fatty acids (11.38%) (USDA 2016). In response to the WHO recommendations, and considering the current consumer demand for healthier meat products, especially the most consumed worldwide, such as mortadella (Berasategi et al. 2011), a strategy to improve the nutritional quality of meat products is to change their lipid profile through the substitution of animal fat by other fat sources. An extensive variety of different non-meat fats, such as vegetable and marine oils, have been used as partial fat substitutes (mainly beef and pork fat) in various meat products (Jiménez-Colmenero 2007).

However, the replacement of animal fat by different non-meat fats represents a major challenge in the development of meat products in terms of both sensory and technological aspects. Fat plays an important role in the manufacturing process as well as in the final texture of mortadella (Saldaña et al. 2015a). According to Saldaña et al. (2015b), sensory and instrumental texture properties of mortadella were affected by the level of animal fat in the formulation, since lower hardness was observed in products with higher fat content. Fat reduction promoted changes in the mortadella microstructure, generating products with a more disorganized structure. Consequently, the lower the animal fat amount in the formulation, the harder the mortadella.

Different technological alternatives for animal fat replacement have been studied (Jiménez-Colmenero 2007). Liquid oils have different physicochemical characteristics compared to animal fats that are commonly used. Liquid oils are liquid at room temperature or even under refrigeration. In this case, the stabilization of oil in pre-emulsions before incorporating into the final product is a promising option in order to avoid liquid loss and produce a more stable meat matrix. Most studies using pre-emulsion as animal fat substitute were based on pre-emulsions composed of a single type of fat or hydrocolloid (Delgado-Pando et al. 2010; Cofrades et al. 2013). However, to the best of our knowledge, there are no studies on the influence of a pre-emulsion of oils and stabilizers on the texture and lipid profile of low-fat mortadella.

In such studies, involving a considerable number of variables, the use of a sequence of two or more experimental designs is an important and useful tool for the optimization process (Granato and Ares 2014). In previous studies, Marchetti et al. (2014) used the response surface methodology (RSM) to optimize milk proteins and carrageenan contents in low-fat sausages with pre-emulsified fish oil. In another study, Pappa et al. (2000) used RSM to determine the optimum salt and pectin level, when olive oil replaced pork back fat for the production of highly acceptable low-fat frankfurters.

In this context, this study aims to optimize the fatty acid profile of the lipids of the pre-emulsion used as fat replacer and the instrumental hardness of low-fat mortadella.

## Materials and methods

### Materials

The ingredients used in the formulations were beef meat (forequarter), pork meat (shoulder), and pork back fat that were obtained from a local market (Piracicaba, Brazil). The oils used were olive oil (Olivas do Sul, Brazil), canola, oil (Cargill, Brazil) and fish oil (Carlson, Norway). The materials used for stabilization of oil-in-water pre-emulsion, such as sodium alginate, milk protein concentrate, condiments and other additives were donated by Ibrac® (Rio Claro, Brazil). The expandable cellulose casings were donated by Viscofan (São Paulo, Brazil).

### Experimental design, modeling and optimization

A sequential strategy of the experimental design was used, composed of two steps. In the first step, an optimization of the mixture of oils of the pre-emulsion was performed using a simplex-centroid mixture design. The second step carried out optimized the constituents of pre-emulsion using a central composite rotatable design.

#### *First step: lipid mixture*

Firstly, a simplex-centroid mixture design (SCMD) (Bruns et al. 2005) was used to assess the effects of binary and ternary mixtures of olive, canola and fish oils on the n-6/n-3 ratio, polyunsaturated/monounsaturated fatty acid (PUFA/SFA) ratio, and the contents of EPA and DHA. Ten experimental assays were used, in which the independent variables (factors) were olive, canola, and fish oils. The design and the levels for the three independent variables are presented in Table 1. The oil mixtures were designed to produce a healthier lipid formulation, with low amounts of SFA, high proportions of MUFA and PUFA (including long chain n-3 PUFA), n-6/n-3 and PUFA/SFA ratios in agreement with the health recommendations (Delgado-Pando et al. 2010).

Mathematical modeling was then carried out considering the SCMD responses as a function of the independent variables (lipid source). After which, an analysis of

**Table 1** Simplex-centroid mixture design used to evaluate the effect of canola, olive and fish oils on the polynsaturated/saturated (PUFA/SFA) and omega-3/omega-6 (n-6/n-3) ratios, and eicosapentaenoic (EPA) and docosahexaenoic (DHA) fatty acids contents

| Experimental assay | Independent variables (original and coded) | | | Responses variables | | | |
|---|---|---|---|---|---|---|---|
| | Canola oil (g 100 $g^{-1}$) | Olive oil (g 100 $g^{-1}$) | Fish oil (g 100 $g^{-1}$) | PUFA/SFA | n-6/n-3 | EPA | DHA |
| 1 | 100 (1) | 0 (0) | 0 (0) | 5.48 | 1.90 | 0 | 0 |
| 2 | 0 (0) | 100 (1) | 0 (0) | 1.26 | 4.87 | 0 | 0 |
| 3 | 0 (0) | 0 (0) | 100 (1) | 1.36 | 0.25 | 17.62 | 7.73 |
| 4 | 50 (0.5) | 50 (0.5) | 0 (0) | 1.70 | 3.93 | 0 | 0 |
| 5 | 50 (0.5) | 0 (0) | 50 (0.5) | 0.77 | 2.64 | 0 | 0 |
| 6 | 0 (0) | 50 (0.5) | 50 (0.5) | 2.73 | 2.15 | 0 | 0 |
| 7 | 33.33 (0.333) | 33.33 (0.333) | 33.33 (0.333) | 1.27 | 1.11 | 3.05 | 3.95 |
| 8 | 66.67 (0.666) | 16.67 (0.167) | 16.67 (0.167) | 0.55 | 2.03 | 0 | 0 |
| 9 | 16.67 (0.167) | 66.67 (0.667) | 16.67 (0.167) | 2.19 | 0.99 | 2.48 | 6.21 |
| 10 | 16.67 (0.167) | 16.67 (0.167) | 66.67 (0.667) | 2.67 | 0.68 | 1.69 | 11.37 |

Coded values are shown in parentheses

variance was carried out to evaluate the statistical significance and the coefficient of determination ($R^2$) of the cubic equation in order to select the most appropriate model for the experiment. The regression model used to estimate the coefficients is shown in Eq. 1.

$$Y = B_1X_1 + B_2X_2 + B_3X_3 + B_{12}X_1X_2 + B_{13}X_1X_3 + B_{23}X_2X_3 + B_{123}X_1X_2X_3 \quad (1)$$

where Y is the predicted response, $B_1$, $B_2$, and $B_3$ are the regression coefficients for the linear effect; $B_{12}$, $B_{13}$, and $B_{23}$ represent the binary interaction of the coefficients, and $B_{123}$ represents the ternary regression coefficient. In the same sense, $X_1$, $X_2$, and $X_3$ represent the canola, olive, and fish oils. An analysis of variance of the model was performed, where the effects and the linear, quadratic, and cubic regression coefficients were calculated. Next, the regression coefficients were used to generate the two-dimensional contour graphic for each variable. The four responses were then overlapped, in order to select the area of interest (Siche et al. 2015; 2016).

*Second step: pre-emulsion components*

Once the mixture of olive, canola, and fish oils was optimized, a central composite rotatable design (CCRD) was used to evaluate the proportion of the pre-emulsion components (lipid mixture, sodium alginate, and milk protein concentrate—independent variables) on the instrumental hardness and springiness of mortadella (dependent variables). A CCRD with three independent variables, two levels ($2^3$) and six axial points ($\alpha = 1.68$) generated seventeen combinations (Table 2), including three replicates of the central point in order to estimate pure error and assess the lack of fit of the proposed models.

The normality of the residues was evaluated by the Anderson–Darling's test and the homogeneity of the variances by the Levene's test. After these analyses, an analysis of variance on the treatments was carried out and when they were significant, the Tukey's test at 5% of significance was carried out. After analyzing the effects, a second order model was adjusted (Eq. 2).

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i=1}^{k} \beta_{ij} X_i X_j \quad (2)$$

where Y is the expected response, $\beta_0$ is the intercept of the model; $\beta_i$, $\beta_{ii}$, and $\beta_{ij}$ represent the linear, quadratic and interaction regression coefficients, respectively. $X_i$ and $X_j$ are the levels of the independent variables, and k is the number of times that each variable was tested ($k = 5$). The analysis of variance associated with the F-test was applied to verify the significance of the models and the factors that were not significant were removed from the model. The data were then re-fitted for the significant factors only. The lack of fit and the coefficient of determination ($R^2$) were considered in the analysis. Finally, after the construction of the models, a simultaneous optimization was carried out using the desirability function (Derringer and Suich 1980). Based on a previous study (Saldaña et al. 2015b), the criteria for the optimization were: for hardness minimum ($> 13$ N) and maximum ($< 16$ N), and for springiness minimum ($< 0.87$), using 60 iterations to render the best mortadella formulation.

*Software*

The mathematical modeling of the SCMD and CCRD was performed using the software Statistica (version 12.0, StatSoft, USA). XLSTAT (version 2015.5, Addinsoft,

**Table 2** Central composite rotatable design used to evaluate the effect of the lipid mixture, sodium alginate and milk protein concentrate of the pre-emulsion on the hardness and springiness of mortadella

| Experimental assay | Independent variables (Original and coded) | | | Response variables | |
|---|---|---|---|---|---|
| | Sodium alginate (g 100 $g^{-1}$) | Milk protein concentrate (g 100 $g^{-1}$) | Lipid mixture (g 100 $g^{-1}$) | Hardness[a] (N) | Springiness[a] |
| 1 | 0.20 (− 1) | 0.20 (− 1) | 14.10 (− 1) | 22.33 ± 0.62[d] | 0.91 ± 0.00 |
| 2 | 0.80 (+ 1) | 0.20 (− 1) | 14.10 (− 1) | 21.19 ± 1.09 [cd] | 0.92 ± 0.00 |
| 3 | 0.20 (− 1) | 0.80 (+ 1) | 14.10 (− 1) | 15.07 ± 0.85[ab] | 0.91 ± 0.00 |
| 4 | 0.80 (+ 1) | 0.80 (+ 1) | 14.10 (− 1) | 12.03 ± 0.34[a] | 0.92 ± 0.00 |
| 5 | 0.20 (− 1) | 0.20 (− 1) | 25.90 (+ 1) | 18.63 ± 0.34[bcd] | 0.91 ± 0.00 |
| 6 | 0.80 (+ 1) | 0.20 (− 1) | 25.90 (+ 1) | 15.60 ± 1.08[ab] | 0.92 ± 0.00 |
| 7 | 0.20 (− 1) | 0.80 (+ 1) | 25.90 (+ 1) | 15.45 ± 0.96[ab] | 0.92 ± 0.00 |
| 8 | 0.80 (+ 1) | 0.80 (+ 1) | 25.90 (+ 1) | 15.02 ± 0.75[ab] | 0.92 ± 0.00 |
| 9 | 0.00 (− 1.68) | 0.50 (0) | 20.00 (0) | 14.49 ± 0.42[ab] | 0.91 ± 0.00 |
| 10 | 1.00 (+ 1.68) | 0.50 (0) | 20.00 (0) | 14.93 ± 0.50[ab] | 0.91 ± 0.00 |
| 11 | 0.50 (0) | 0.00 (− 1.68) | 20.00 (0) | 17.04 ± 0.59[bc] | 0.91 ± 0.00 |
| 12 | 0.50 (0) | 1.00 (+ 1.68) | 20.00 (0) | 18.01 ± 1.13[bcd] | 0.92 ± 0.00 |
| 13 | 0.50 (0) | 0.50 (0) | 10.00 (− 1.68) | 15.52 ± 1.74[ab] | 0.92 ± 0.01 |
| 14 | 0.50 (0) | 0.50 (0) | 30.00 (+ 1.68) | 17.46 ± 1.18[bc] | 0.92 ± 0.01 |
| 15 | 0.50 (0) | 0.50 (0) | 20.00 (0) | 15.64 ± 0.97[ab] | 0.91 ± 0.00 |
| 16 | 0.50 (0) | 0.50 (0) | 20.00 (0) | 15.44 ± 1.12[ab] | 0.92 ± 0.00 |
| 17 | 0.50 (0) | 0.50 (0) | 20.00 (0) | 18.99 ± 0.32[bcd] | 0.92 ± 0.00 |
| *P* (Normality)[b] | | | | 0.28 | 0.07 |
| *P* (Homogeneity)[c] | | | | 0.22 | 0.75 |
| *P* (Anova)[d] | | | | <0.01 | 0.17 |

Coded values are shown in parentheses

[a]Values expressed as mean ± standard error of the mean (n = 5)

[b]Probability values obtained by the Anderson–Darling's test (normality of residuals)

[c]Probability values obtained by the Levene's test (homogeneity of variances)

[d]Probability values obtained by one-way ANOVA

USA) was used in the study of assumptions of the analysis of variance (ANOVA) and the software Design Expert (version 7.0, Stat Ease, USA) was used to obtain the overlapping of the graphics in the SCMD.

## Preparation of oil-in-water pre-emulsions

A mixture of olive (30%), canola (50%), and fish (20%) oils was used. This specific mixture was obtained from the previous optimization using a SCMD. Seventeen oil-in-water pre-emulsions were prepared for addition to the mortadella, following a CCRD (Table 2). The pre-emulsions were prepared by stirring the sodium alginate and milk protein concentrate (these components were established from the study of Saldaña et al. (2015a) with ultrapure water using a magnetic stirrer (IKA, model RH basic 1) at 500 rpm and 60 °C, until complete dissolution. After cooling to room temperature, the solution was emulsified with the mixture of oils at 500 rpm for 10 min at 30 °C (Delgado-Pando et al. 2010; Marchetti et al. 2014). The quantity of water in each combination varied according to the other components (hydrocolloids and oils) in order to obtain a sum of 100%.

### *Manufacture of mortadella*

The mortadella was manufactured at the Meat Processing Plant of the Escola Superior de Agricultura "Luiz de Queiroz" (ESALQ), Universidade de São Paulo (USP) according to a CCRD (Table 2). For the development of mortadella with partial fat replacement by pre-emulsion, the study of Saldaña et al. (2015b) was considered, which formulated traditional mortadella (full fat) with 16% fat based on the fat content that is commonly found in Brazilian products. Thus, in this study, mortadellas were formulated with 50% animal fat (i.e. 8%) and 8% pre-emulsion. The ingredients in the basic formulation (g 100 $g^{-1}$) were: 36 g beef forequarter, 30 g pork shoulder, 8 g pork back fat, 0.8 g salt, 1 g seasonings, 0.35 g curing salt (90% salt, 6% sodium nitrite, and 4% sodium nitrate),

0.35 g sodium tripolyphosphate, 0.2 g garlic paste without salt, 2 g maltodextrin, and 0.35 g of antioxidant (sodium erythorbate). The mortadella was manufactured as described by Saldaña et al. (2015a). Meat (without visible fat and connective tissue) and pork back fat (4 °C) were ground using a 20-mm plate (Hobart 4B22-2, USA). Beef and pork meat were homogenized for 1 min in a chilled cutter (2 °C) (Kramer Grebe®, model T160, Germany). Salt, curing salt, phosphates, spices, garlic paste and maltodextrin were added to the meat along with 50% of the ice, required to reduce the mixture temperature to 0 °C and prevent protein denaturation. Next, animal fat (pork back fat) and oil-in-water pre-emulsions were added to the formulations. The remaining 50% of ice was then added to the mixture and finally, the antioxidants. The final mixture temperature was 12.5 ± 0.8 °C. The batter was stuffed into expandable cellulose casings with a 72 mm radius using a hydraulic stuffer (Frigomaq, model FMQ, Brazil). The thermal treatment was performed in an oven (Verinox, model Junior, Italy), and occurred in two steps. In the first step, the oven temperature was kept at 50 °C for 1 hour, with indirect steam and an open chim-ney. In the second step, the temperature was kept at 60 °C for another hour, using indirect steam and a closed chimney, and it was halted when the geometric center of the product reached 73 °C. After the cooking process, the products were cooled using water spray (20 °C) for 30 min before weighing and vacuum packing (Saldaña et al. 2015b).

## Analytical methods

### *Fatty acid profile of the lipid mixtures*

Fatty acid methyl esters were prepared according to the method described by Aldai et al. (2012). Aliquots (~ 10 mg) of the oils were methylated using a 0.5 N methanolic solution of sodium methoxide ($NaOCH_3$) that reacted with the sample at 50 °C for 15 min. Then, a methanolic solution of hydrochloric acid (methanol:hydrochloric acid, 95:5, v/v) was added to the sample and it was heated to 80 °C for 30 min.

The fatty acid methyl esters (FAME) were analyzed using a gas chromatograph equipped with a flame ionization detector (Shimadzu, model 2010—Plus gas chromatograph, Japan) and a fused silica capillary column (Supelco, model SP—2560, USA, 100 m × 0.25 mm i.d. × 0.20 μm film thickness). A heating ramp was used, according to the following temperature program: (1) an increase from 100 to 170 °C at 2 °C $min^{-1}$ (kept for 15 min); (2) an increase to 180 °C at 0.5 °C $min^{-1}$; (3) an increase to 200 °C at 10 °C $min^{-1}$; (4) an increase to 230 °C at 2 °C $min^{-1}$; (5) a final hold at 230 °C for 13 min. The temperatures of the injector and detector were kept at 240 °C. Nitrogen was used as the carrier gas at a flow rate of 1.5 mL $min^{-1}$, in a split injection mode, at a 1:30 ratio. Samples (1 μL) were injected and individual FAME peaks were identified by comparison of their retention times with those of the standard (FAME Mix C4-C24, Supelco, USA). The FAME contents were expressed as percentage (%) of the total FAME quantified.

### *Texture measurements of mortadella*

The Texture Profiling Analysis (TPA) was determined to measure the hardness and springiness of the product. The analysis was carried out at room temperature (25 ± 2 °C), with a Texture Analyzer (model TA-xT2i, Texture Technologies Corp., USA) as described by Bourne (2002). Six cylinders (2 cm diameter × 2 cm height) were taken from the samples (Saldaña et al. 2015a, b), and subjected to a two-cycle compression test. The samples were compressed to 30% of their original height with a P-35 probe (long shaft, regular base) at a speed of 2 mm $s^{-1}$. The following parameters were determined: hardness (maximum force during the first cycle of compression), and springiness (the height at which the food recovers during the time that elapses between the end of the first cycle and the beginning of the second cycle) (Horita et al. 2011; de Almeida et al. 2014).

## Results and discussion

### Optimization of the lipid mixture

### *Fatty acid profile of oils*

The oils used in this study were selected in order to obtain a healthy fatty acid profile (i.e. low amounts of SFA, high proportions of MUFA and PUFA, including long chain n-3 PUFA). Olive oil stands out in terms of MUFA content, fish oil is considered the primary source of long chain n-3 PUFA, and canola oil presents the lowest level of SFA among the most common vegetable oils, in addition to presenting high MUFA levels, and intermediate PUFA levels (USDA 2016).

The lipid profile of the three oils studied showed that the vegetable oils had oleic acid as the major fatty acid of their compositions (> 50%), followed by linoleic acid, representing 25.68 and 18.67% of the fatty acids of canola oil and olive oil, respectively (Table 3). For n-3 fatty acids, canola oil showed the highest amount of linolenic acid and since EPA and DHA are marine-derived n-3 fatty acids, they were found only in the fish oil. The increase in the consumption of n-3 fatty acids, such as linolenic acid, EPA

and DHA, has been associated with potential health effects, especially regarding cardiovascular diseases.

For the PUFA/SFA ratio, canola oil showed higher values when compared with the other components, indicating a high PUFA content and a low SFA content. The binary mixture composed of olive and fish oils showed the second highest value, and the ternary mixtures encoded as experimental assay 9 and 10, both formulated with 16.67% canola oil, showed values higher than 2 (Table 1). The values of the other combinations were all lower than 2, but still higher than 0.4, which is the recommended threshold to keep a healthy diet (Wood et al. 2004), since lower ratios may increase the incidence of cardiovascular disease (Cifuni et al. 2004).

According to the results reported by Saldaña et al. (2015a), the PUFA/SFA ratio alone is not enough to indicate the nutritional quality of a fat, since the PUFA analyzed in foods usually contain far more n-6 than n-3 in their compositions, an imbalance that is not recommended for a healthy diet. In this context, it is also important to consider the n-6/n-3 ratio within the PUFAs For the prevention of cardiovascular disease, the recommendation is to reduce this ratio to less than 4 (Salcedo-Sandoval et al. 2014). The low values are therefore related to healthy diets, as they indicate a higher amount of n-3 fatty acids. In this study, the n-6/n-3 ratio ranged from 0.25 to 4.87. Except for the experimental assay 2 (100% olive oil), none of the lipid compositions had values higher than 4, indicating that a mixture of them, with the appropriate proportions, could improve the lipid quality of a food matrix, especially meat products that usually have high n-6/n-3 ratios (Wood et al. 2004, 2008). As expected, fish oil (experimental assay 3) had the lowest n-6/n-3 ratio, indicating a nutritional advantage compared to the other oils evaluated. This low value also showed that the low PUFA found in this oil did not influence the n-6/n-3 ratio, probably due to its higher n-3 amount. Canola oil also showed a low n-6/n-3 ratio, which was lower than the value found for olive oil. For ternary combinations, the presence of fish oil (experimental assay 10) helped to reduce the n-6/n-3 ratio. Due to the lipid quality of fish oil, it presents high potential to be used for lipid profile improvement of meat products. However, it is important to take into account the oxidative stability and characteristic flavor, which may decrease consumer acceptance of the product (Valencia et al. 2008). Thus, obtaining a suitable lipid mixture from the nutritional viewpoint, which does not provoke a reduction in consumer acceptance, is a critical factor. To this effect, we aimed to replace the least amount of animal fat, thereby improving the lipid profile of the mortadella, without affecting the sensory properties of product.

**Table 3** Fatty acid profile (%) of canola, olive and fish oils used in the study

| Fatty acid | Canola oil | Olive oil | Fish oil |
|---|---|---|---|
| C14:0 | – | – | 6.90 |
| C15:0 | – | – | 0.45 |
| C16:0 | 3.85 | 15.86 | 14.47 |
| C16:1 | – | 5.17 | 10.95 |
| C17:0 | – | – | 1.36 |
| C17:1 | – | – | 1.95 |
| C18:0 | 1.94 | 2.04 | 2.91 |
| C18:1 n-9t | – | – | 1.92 |
| C18:1 n-9c | 52.33 | 52.12 | 10.75 |
| C18:2 n-6c | 25.68 | 18.67 | 5.29 |
| C18:3 n-6 | – | – | 0.96 |
| C18:3 n-3 | 13.51 | 3.83 | 2.78 |
| C20:0 | – | – | 0.87 |
| C20:2 | – | – | 4.48 |
| C20:3 n-6 | – | – | 0.65 |
| C20:4 n-6 | – | – | 0.50 |
| C20:5 n-3 | – | – | 17.62 |
| C21:0 | – | – | 0.70 |
| C22:0 | 1.37 | – | 0.98 |
| C22:1 n-9 | – | – | 2.36 |
| C22:2 | – | – | 0.61 |
| C22:6 n-3 | – | – | 7.73 |
| C23:0 | – | – | 1.27 |
| SFA | 7.16 | 17.90 | 29.90 |
| TRANS | – | – | 1.92 |
| MUFA | 52.33 | 57.28 | 26.02 |
| PUFA | 39.20 | 22.50 | 40.62 |
| n-6/n-3 | 1.90 | 4.87 | 0.25 |
| PUFA/SFA | 5.48 | 1.26 | 1.36 |

Regarding the content of the long-chain n-3 fatty acids, both EPA and DHA were only present in the fish oil and in the ternary combinations in which it was added. There is strong evidence to suggest that the intake of EPA and DHA is related to the prevention of coronary heart disease. The potential mechanisms for the cardioprotective effects include anti-arrhythmic, anti-thrombotic and anti-inflammatory effects, reduction in blood pressure, improved endothelial function, and retarded growth of atherosclerotic plaques (Connor 2000; Geleijnse et al. 2002). These beneficial health effects show the importance of the supplementation of foods with long-chain n-3 fatty acids.

The fatty acid profile allowed identifying the potential of the different combinations and proportions of the oils studied here. Thus, the next step of the study was to model the dependent variable as a function of the three components of the mixtures, using a mixture design of the response surface methodology. The representation of the contour surfaces, using the special cubic model, is shown in Fig. 1.

The special cubic equation was selected to model the four dependent variables as a function of the three lipid components due to its high coefficient of determination ($R^2$). Thus, it can be seen in Fig. 1 that the PUFA/SFA ratio increases when there is canola oil in the lipid mixture. It also shows that the higher the fish oil in the composition, the lower the n-6/n-3 ratio. Additionally, EPA and DHA levels increase as fish oil increases. The overlaying of graphic surfaces of the four response variables revealed the importance of fish oil, since the addition of a small amount of fish oil was effective for improving the nutritional quality of the mixture. Based on these responses, a mixture of oils within the optimal area (Fig. 1 - orange filled area of the central triangle) was selected, consisting of 50% canola oil, 30% olive oil and 20% fish oil.

The optimized lipid mixture was modeled and presented the following values: PUFA/SFA = 2.28; n-6/n-3 = 2.30; EPA = 0.81; DHA = 1.66, which are within the recommendations of a healthy diet. The optimized mixture comprised of only 20% fish oil in order to avoid alterations in the sensory characteristics of the product, in agreement with Jiménez-Colmenero et al. (2013).

Once the composition of the lipid mixture was optimized, the next step of the study was to optimize instrumental hardness and springiness of mortadella, through the partial replacement of animal fat by a pre-emulsion composed by the lipid mixture, sodium alginate and milk protein concentrate, using the Texture Profile Analysis.

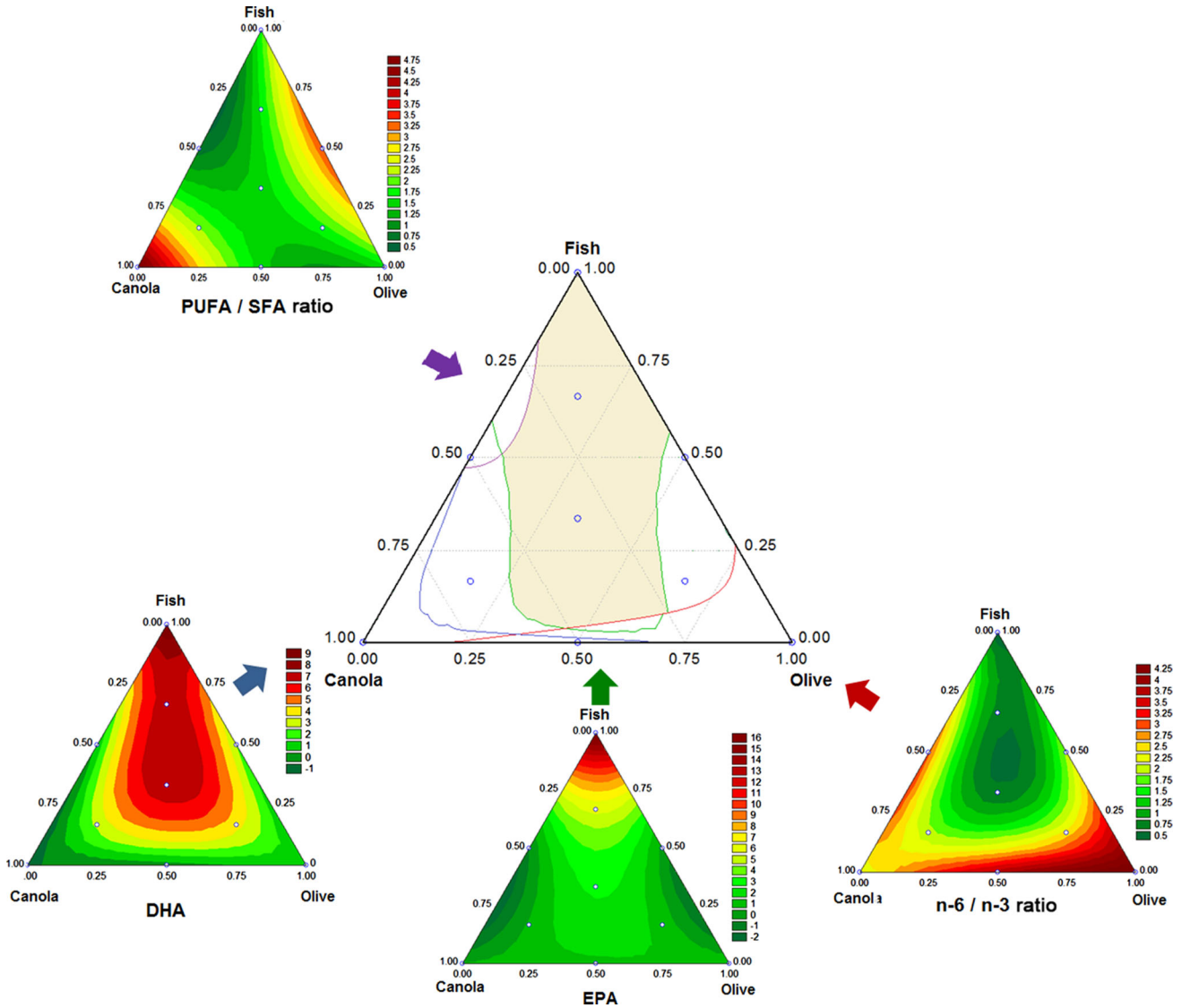


**Fig. 1** Overlapped contour surfaces of the four response variables evaluated in the lipid mixtures

### Optimization of pre-emulsion

According to Saldaña et al. (2015b), fat reduction of mortadella results in an increase in instrumental and sensory hardness. Therefore, the present study aims to optimize instrumental hardness and springiness of the product. To optimize these parameters, the study of Saldaña et al. (2015b) was taken into account. The authors evaluated the microstructure and instrumental and sensory texture of traditional and light mortadella and found that hardness and springiness of Brazilian commercial mortadella ranged from 12.88 to 16.24 and from 0.86 to 0.87, respectively.

The results of instrumental hardness and springiness of mortadella with pre-emulsion as animal fat replacer are shown in Table 2. The two dependent variables show normality of residues ($P > 0.05$) and homogeneity of variances ($P > 0.05$). There was a significant effect of the treatments on instrumental hardness ($P < 0.05$), which ranged from 12.13 to 22.34 N, while springiness was not significantly affected ($P > 0.05$). Since there was no significant difference among treatments for springiness (the factors studied, within the levels evaluated, did not influence this response), it was not mathematically modeled. The present work shows positive results regarding mortadella springiness. Marchetti et al. (2014) evaluated fish oil, milk protein and carrageenan in low-fat meat sausages, and Zouari et al. (2012) studied whey powder and carrageenan in turkey meat sausage and found higher springiness in treatments with the addition of a hydrocolloid and milk protein.

Figure 2 shows hardness variation regarding the factors evaluated in this research. Instrumental hardness of the product changed according to the concentration of emulsion stabilizers (sodium alginate and milk protein concentrate) in the pre-emulsion. As the concentration of both components increased, hardness decreased. This hardness decrease may be attributed to a water hold-ing capacity caused by the addition of the hydrocolloid, resulting in products with lower resistance to chewing. Similarly Pappa et al. (2000) reported that low-fat frankfurters produced with high pectin levels were very soft. However, Marchetti et al. (2014) studied sausages with the addition of fish oil, milk proteins and carrageenan, and found that carrageenan and milk protein increased product hardness. Since hardening and softening have been observed when a stabilizer is added to low-fat meat products, these results may be due to the amount and characteristics of each ingredient used, as well as the type of meat product studied.

Due to this behavior, it is important to model and find the optimal concentration of each component in order to obtain a product with hardness values between 13 and 16 N. When this texture parameter was evaluated as a function of the emulsion stabilizers and the lipid mixture,

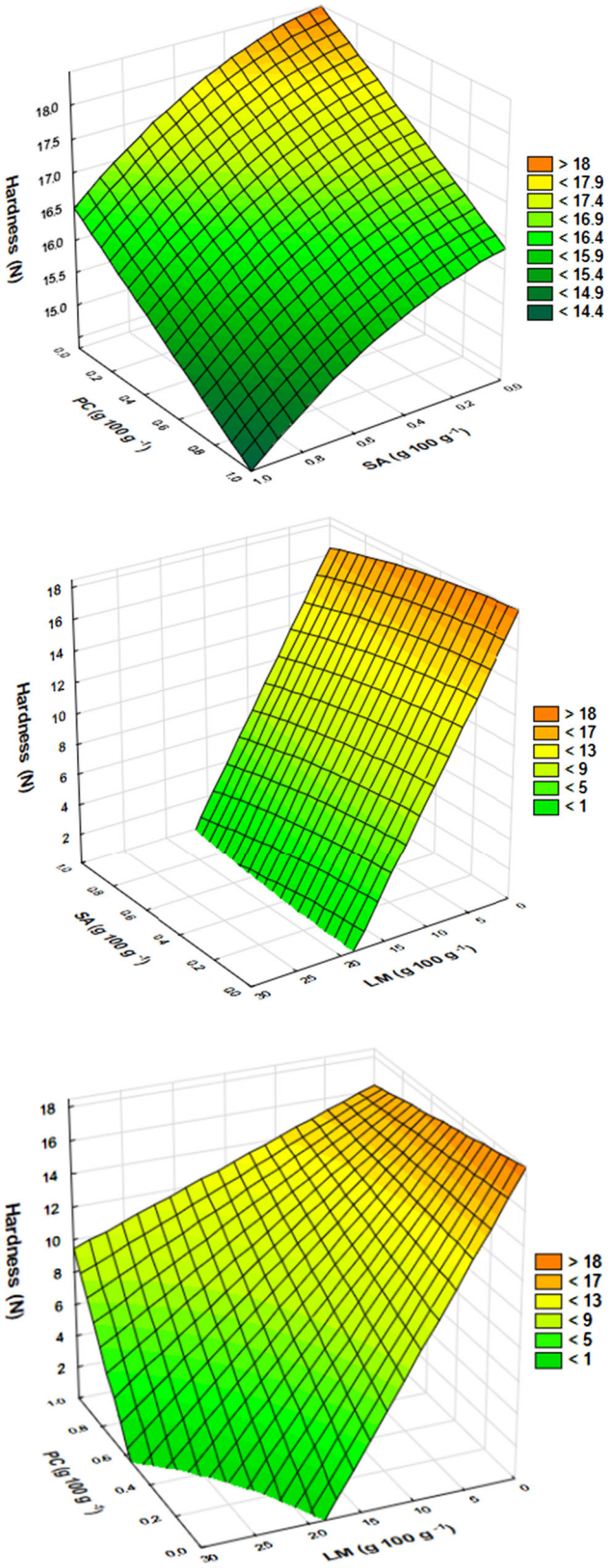


**Fig. 2** Effect of sodium alginate (SA), milk protein concentrate (PC) and lipid mixture (LM) on instrumental hardness of mortadella

an increase in the concentration of the oils resulted in a decrease in hardness. This is in agreement with the findings of other studies that have shown softer texture as a result from the direct incorporation of oils into meat products (Álvarez et al. 2012). This is most likely due to the liquid character of oil compared to the solid character of animal fat (Pelser et al. 2007). Considering the influence of the oil concentrations on hardness, it is also important to find the optimum proportion of the lipid mixture of the pre-emulsion.

According to the estimated effects of instrumental hardness on mortadella, linear and quadratic effects, as well as the interaction between them, were significant for some of the independent variables studied (SA: sodium alginate, PC: milk protein concentrate, LM: lipid mixture). The polynomial regression model of the instrumental hardness was significant ($P < 0.05$) and is described in Eq. 3.

$$Hardness = 18.48 - 0.64 \cdot SA - 1.36 \cdot SA^2 - 2.16 \cdot PC - 1.00 \cdot LM + 0.77 \cdot PC \cdot LM \quad (3)$$

The coefficient of determination ($R^2$) of the model was 0.93, indicating that the variability of the responses can be satisfactorily explained by the model. In practice, high $R^2$ values are considered reasonable indicators of suitability of regression models, describing the influence of independent variables (Baş and Boyacı 2007). The experimental error of 0.14% shows that the experiment was well conducted, and indicates that the obtained model is reliable. The analysis of variance showed that the model did not present lack of fit ($P = 0.08$), therefore, the model was considered predictive.

The simultaneous optimization of instrumental hardness and springiness was performed using the "desirability function", considering the optimal responses of the experimental data, with a small range of variation for each variable (Derringer and Suich 1980). This function searches for the best option within the ranges of values showed as optimal. From this, the desirability index obtained was 1, and the software indicated the following optimal composition: 0.51% sodium alginate, 0.50% milk protein concentrate and 30% of the lipid mixture.

## Conclusion

The response surface methodology was efficient to perform the modeling of the lipid mixture and instrumental hardness of mortadella. The equations developed may be used for predictive purposes. The overlapping of contour surfaces was used to select the proportions of each lipid source, maximizing the PUFAs, specifically the long-chain n-3 fatty acids, such as EPA and DHA. Thus, the optimized proportions of the oils were 20% fish oil, 30% olive oil, and 50% canola oil. The desirability function was used to optimize the components of the pre-emulsion according to instrumental hardness and springiness. The optimized pre-emulsion was composed of 0.51% sodium alginate, 0.50% milk protein concentrate and 30% of the lipid mixture. Our results confirmed that fat substitution by pre-emulsions modified instrumental hardness. This study can be considered a starting point for further studies that seek to optimize the sensory properties of low-fat mortadella.

**Acknowledgements** Erick Saldaña thanks the "Ministerio de Educación del Perú" for the scholarship granted by the program "Programa Nacional de Becas y Crédito Educativo" (PRONABEC), Peru. The authors are grateful to the São Paulo Research Foundation (FAPESP), for funding the Project No 2013/05359-7.

## References

Aldai N, Kramer JKG, Cruz-Hernandez C, Santercole V, Delmonte P, Mossaba MM, Dugan MER (2012) Appropriate extraction and methylation techniques for lipids analysis. In: Cherian G, Poureslami R (eds) Fat and fatty acids in poultry nutrition and health. Context, Packington, pp 249–278

Álvarez D, Xiong YL, Castillo M, Payne FA, Garrido MD (2012) Textural and viscoelastic properties of pork frankfurters containing canola-olive oils, rice bran, and walnut. Meat Sci. https://doi.org/10.1016/j.meatsci.2012.03.012

Baş D, Boyacı İH (2007) Modeling and optimization I: usability of response surface methodology. J Food Eng. https://doi.org/10.1016/j.jfoodeng.2005.11.024

Berasategi I, Legarra S, García-Íñiguez de Ciriano M, Rehecho S, Calvo MI, Cavero RY, Navarro-Blasco I, Ansorena D (2011) "High in omega-3 fatty acids" bologna-type sausages stabilized with an aqueous-ethanol extract of *Melissa officinalis*. Meat Sci. https://doi.org/10.1016/j.meatsci.2011.02.035

Bourne M (2002) Food texture and viscosity: concept and measurement, 2nd edn. Academic Press, Florida

Bruns RE, Neto BB, Scarminio IS (2005) Simplex optimization. In: Bruns RE, Neto BB, Scarminio IS (eds) Statistical design—chemometrics. Elsevier, Amsterdam, pp 365–380

Cifuni GF, Napolitano F, Riviezzi AM, Braghieri A, Girolami A (2004) Fatty acid profile, cholesterol content and tenderness of meat from Podolian young bulls. Meat Sci. https://doi.org/10.1016/j.meatsci.2003.10.017

Cofrades S, Antoniou I, Solas MT, Herrero AM, Jiménez-Colmenero F (2013) Preparation and impact of multiple (water-in-oil-in-water) emulsions in meat systems. Food Chem. https://doi.org/10.1016/j.foodchem.2013.02.097

Connor WE (2000) Importance of n-3 fatty acids in health and disease. Am J Clin Nutr 71:171S–175S

de Almeida MA, Villanueva NDM, Gonçalves JR, Contreras-Castillo CJ (2014) Quality attributes and consumer acceptance of new ready-to-eat frozen restructured chicken. J Food Sci Technol. https://doi.org/10.1007/s13197-014-1351-5

Delgado-Pando G, Cofrades S, Ruiz-Capillas C, Jiménez-Colmenero F (2010) Healthier lipid combination as functional ingredient influencing sensory and technological properties of low-fat

frankfurters. Eur J Lipid Sci Technol. https://doi.org/10.1002/ejlt.201000076
Derringer G, Suich R (1980) Simultaneous optimization of several response variables. J Qual Technol 12:214–219
Geleijnse JM, Giltay EJ, Grobbee DE, Donders AR, Kok FJ (2002) Blood pressure response to fish oil supplementation: metaregression analysis of randomized trials. J Hypertens. https://doi.org/10.1097/00004872-200208000-00010
Granato D, Ares G (2014) Mathematical and statistical methods in food science and technology. Wiley, Chichester
Horita CN, Morgano MA, Celeghini RMS, Pollonio MAR (2011) Physico-chemical and sensory properties of reduced-fat mortadella prepared with blends of calcium, magnesium and potassium chloride as partial substitutes for sodium chloride. Meat Sci. https://doi.org/10.1016/j.meatsci.2011.05.010
Jiménez Colmenero F (2000) Relevant factors in strategies for fat reduction in meat products. Trends Food Sci Technol. https://doi.org/10.1016/S0924-2244(00)00042-X
Jiménez-Colmenero F (2007) Healthier lipid formulation approaches in meat-based functional foods. Technological options for replacement of meat fats by non-meat fats. Trends Food Sci Technol. https://doi.org/10.1016/j.tifs.2007.05.006
Jiménez-Colmenero F, Triki M, Herrero AM, Rodríguez-Salas L, Ruiz-Capillas C (2013) Healthy oil combination stabilized in a konjac matrix as pork fat replacement in low-fat, PUFA-enriched, dry fermented sausages. LWT Food Sci Technol. https://doi.org/10.1016/j.lwt.2012.10.016
Marchetti L, Andrés SC, Califano AN (2014) Low-fat meat sausages with fish oil: optimization of milk proteins and carrageenan contents using response surface methodology. Meat Sci. https://doi.org/10.1016/j.meatsci.2013.11.004
Pappa I, Bloukas J, Arvanitoyannis I (2000) Optimization of salt, olive oil and pectin level for low-fat frankfurters produced by replacing pork backfat with olive oil. Meat Sci. https://doi.org/10.1016/S0309-1740(00)00024-3
Pelser WM, Linssen JPH, Legger A, Houben JH (2007) Lipid oxidation in n-3 fatty acid enriched Dutch style fermented sausages. Meat Sci. https://doi.org/10.1016/j.meatsci.2006.06.007
Reddy BS (2002) Types and amount of dietary fat and colon cancer risk: prevention by omega-3 fatty acid-rich diets. Environ Health Prev Med. https://doi.org/10.1265/ehpm.2002.95
Salcedo-Sandoval L, Cofrades S, Ruiz-Capillas C, Jiménez-Colmenero F (2014) Effect of cooking method on the fatty acid content of reduced-fat and PUFA-enriched pork patties formulated with a konjac-based oil bulking system. Meat Sci. https://doi.org/10.1016/j.meatsci.2014.07.034
Saldaña E, Behrens JH, Serrano JS, Ribeiro F, de Almeida MA, Contreras-Castillo CJ (2015a) Microstructure, texture profile and descriptive analysis of texture for traditional and light mortadella. Food Struct. https://doi.org/10.1016/j.foostr.2015.09.001
Saldaña E, Lemos ALSC, Selani MM, Spada FP, Almeida MAD, Contreras-Castillo CJ (2015b) Influence of animal fat substitution by vegetal fat on Mortadella-type products formulated with different hydrocolloids. Sci Agric 72:495–503
Siche R, Falguera V, Ibarz A (2015) Use of response surface methodology to describe the combined effect of temperature and fiber on the rheological properties of orange juice. J Texture Stud. https://doi.org/10.1111/jtxs.12112
Siche R, Ávalos C, Arteaga H, Saldaña E, Vieira TMFS (2016) Antioxidant capacity of binary and ternary mixtures of orange, grape, and starfruit juices. Curr Nutri Food Sci 12:65–71
USDA (2016) National nutrient database for standard reference. https://ndb.nal.usda.gov/ndb/search. Accessed 28 July 2016
Valencia I, O'Grady MN, Ansorena D, Astiasarán I, Kerry JP (2008) Enhancement of the nutritional status and quality of fresh pork sausages following the addition of linseed oil, fish oil and natural antioxidants. Meat Sci. https://doi.org/10.1016/j.meatsci.2008.04.024
WHO (2003) Diet, nutrition and the prevention of chronic diseases. World Health Organization technical report series 916 http://apps.who.int/iris/bitstream/10665/42665/1/WHO_TRS_916.pdf. Accessed 30 July 2016
Wood JD, Richardson RI, Nute GR, Fischer AV, Campo MM, Kasapidou E, Sheard PP, Enser M (2004) Effects of fatty acids on meat quality: a review. Meat Sci. https://doi.org/10.1016/S0309-1740(03)00022-6
Wood JD, Enser M, Fisher AV, Nute GR, Sheard PR, Richardson RI, Hughes SI, Whittington FM (2008) Fat deposition, fatty acid composition and meat quality: a review. Meat Sci 78:343–358
Zouari N, Ayadi MA, Hadj-Taieb S, Frikha F, Attia H (2012) Whey powder, ι-carrageenan, and fat interactions and their influence on instrumental texture and sensory properties of turkey meat sausage using a mixture design approach. Int J Food Prop. https://doi.org/10.1080/10942912.2010.517885
Zyriax BC, Windler E (2000) Dietary fat in the prevention of cardiovascular disease—a review. Eur J Lipid Sci Technol. https://doi.org/10.1002/(SICI)1438-9312(200005)102:5<355::AID-EJLT355>3.0.CO;2-3